Structure-based Design of Compounds that Inhibit Detrimental Cytotoxic T Lymphocyte Responses

Introduction

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Background of the Invention

Computational chemistry and molecular modeling can be used to study the surface contacts of receptor-mediated interactions as well as serve as a means to develop small molecule antagonists. Contact regions of a protein's surface are comprised of a pattern of well-defined ridges and channels. The ridges are relatively polar with high electropotential and flexibility in the unbound state. The channels, on the other hand, have a low electropotential and are relatively rigid. Recent biophysical studies suggest that these channels are protected by a shell of water molecules (Sidorova, N.Y. and Rau, D.C., Proc. Natl Acad. Sci. 1996 93(22):12272-12277; Vossen et al. Biochemistry 1997 36(39):11640-11647; Cheng, Y.K. and Rossky, P.J. Nature 1998 392(6677):696-699; Clackson et al. J. Mol. Biol. 1998 277(5):1111-1128 and Pardanani et al. J. Mol. Biol. 1998 248(3):729-739). The flexible, polar ridges that flank the channels in the binding site are used to create a "handshake" with another protein prior to establishing more extensive

contacts and dispersing the ordered water molecules. The result is a high affinity complex. Thus, the small ridges act as guide to bring the proteins together.

Synthetic mimicry of the ridge has been used to create molecules capable of blocking the initial handshake which occurs prior to the high affinity interaction. For example, analogs have been designed from the surface of blood coagulation factor XI to inhibit its binding to high molecular weight kininogen (Baglia et al. J. Biol. Chem. 1992 265:4247-4252). Blood coagulation factor XIa has also been used as a template to design small analogs that potentially compete with its binding to activated platelets. The IGF-1 protein surface has also been used to engineer analogs capable of inhibiting IGF-1 dependent growth of cells derived from a prostate carcinoma (Pietrzkowski et al. Cancer Research 1993 52:6447-6451). The surface of CD4 was used to rationally design mimetics that were able to block CD4-independent T cell stimulation and to significantly inhibit both the severity and the incidence of EAE in rodents (Jameson et al. Nature 1994 368:744-746). IgE has also been used as a template to design effective inhibitors that are capable of blocking its binding to the high affinity Fc receptor so that IgE-induced degranulation of mast cells is inhibited (McDonnell et al. Nature Structural Biology 1996 3:419-425). The domain 5 of kininostatin has also been used to engineer analogs that were able to block its binding to urokinase receptor and to block angiogenesis.

U.S. Patent 5,645,837 describes compounds which interfere with CD8 mediated activity by competing with CD8 in intermolecular interactions that involve CD8 which are associated with cytotoxic T lymphocyte (CTL) activation. These compounds comprise a molecular surface that corresponds to a molecular surface of human CD8 at amino acids 53-56, 60-67 or 53-67 and are able to interact with the same molecules as the CD8 amino acids without producing the same biological

effects as CD8 intermolecular interaction. Peptide analogues SC4 and SC7, which were engineered from CD8 and contain amino acids 54-59 or 63-71 of the CD8 sequence and terminal cysteines, have also been disclosed as capable of disrupting the activation and/or generation phase of CD8 CTLs (Choski et al. Nature Medicine 1998 4:309-314). Small synthetic peptide mimics of the CD8 DE loop have also been shown to possess inhibitory activity on *in vitro* CD8 T cell function (Li et al. J. Biol. Chem. 1998 273(26):16442-5).

The present invention relates to compounds and methods of designing compounds which mimic or interact with surface structures of the CD8 activation complex specific to interaction with Major Histocompatibility Complex class I (MHC I) as a means of disrupting a primary signaling event of detrimental CTL responses.

Summary of the Invention

An object of the present invention is to provide a composition which inhibits a detrimental cytotoxic T lymphocyte response which comprises a compound which mimics or interacts with a surface feature of the CD8/MHC I complex. Unique surface features include, but are not limited to the SHN, KIT, SSK, DEK and RDT surface feature.

Another object of the present invention is to provide a method of producing a compound which inhibits a detrimental cytotoxic T lymphocyte response which comprises identifying a surface feature of CD8 specific to the interaction of CD8 with MHC I; and synthesizing a compound which mimics or interacts with the identified surface feature.

Yet another object of the present invention is to provide a method of inhibiting a detrimental T lymphocyte response in a human by administering to the human a composition which comprises a compound which mimics or interacts with a surface feature of the human CD8/MHC I complex. In one embodiment of the invention, a pharmaceutical

composition comprising a compound with a simple aromatic ring which fits into a hole in the surface of CD8, such as carbobenzoxy arginine, is administered to a human to inhibit a detrimental T lymphocyte response.

Brief Description of the Drawings

Figure 1 is a bargraph showing % cytotoxicity of compounds which mimic or interact with the surface of the CD8 β-chain. Compounds were tested at concentrations of 50, 100 and 200 μM.

Detailed Description of the Invention

There are two arms of the immune system to generate T cell-mediated immunity. The first involves CD4-positive helper T cells which recognize antigen in the context of the Major Histocompatibility Complex class II, while the other involves CD8-positive cytotoxic cells (CTLs) which recognize antigen in the context of Major Histocompatibility Complex class I (MHC I). The CD4-positive T cells provide "helper" functions in mediating both the humoral as well as cellular immune responses. In healthy individuals, the CTL response is intended to kill cells infected with intracellular pathogens, such as viruses, parasites and bacteria.

However, in addition to their protective roles in the body, both the helper T cells as well as CTLs have been implicated in a variety of different pathological situations. For example, human gene therapy is rapidly on its way to becoming a medical reality. There are currently ongoing Phase I clinical trials for gene therapy for treatment of a variety of diseases including cancer, cystic fibrosis, Gaucher's Disease and arthritis. To successfully treat a patient, the engineered cell must be targeted and delivered to the appropriate cells. An efficient gene delivery system has been found to be the adenoviral vector. However, during the process of gene delivery and vector replication, viral

proteins are produced and presented to the host's immune system. In turn, a powerful CTL response is generated that targets and destroys cells containing the newly delivery gene. This CTL response thus limits the effectiveness of gene therapies.

A detrimental CTL response also occurs in patients with insulin-dependent diabetes mellitus (IDDM). While the exact etiology of this disease is unknown, it is known that activated CTLs interact specifically with β-cells of the pancreatic islets to destroy the β cells (Rabinovitch, A. and Suarez-Pinzon, W.L. Biochem Pharmacol. 1998 55(8):1139-1149).

The allospecific responses generated by tissue transplantation are also very difficult to control as the immunological responses governing rejection are both diverse and complicated. It appears that rejection can occur in the absence of both CD4-dependent and CD8-dependent responses. For example, an allogenic skin graft from a mouse genetically devoid of both class I and class II is rapidly rejected by a normal mouse recipient (Grusby et al. Proc. Natl Acad. Sci. USA 1993 90:3913-3917). In spite of the diversity of responses that can lead to graft rejection, multiple studies have demonstrated that CD8-positive lymphocytes are important in early allograft rejection (Rukavina et al. Transplant 1996 61(2):285-291; Allan et al. Annals of Thoracic Surgery 1997 64:1019-1025; He et al. Transplant. Proc. 1998 30:1069-1070; Carpenter et al. Journal of Vascular Surgery 1998 27(3):492-499; and Wong et al. Hepatology 1998 28(2):443-449).

Accordingly, agents are needed which inhibit this detrimental CTL response without affecting the general host immune defense system. One means for therapeutically targeting the detrimental CTL response without interfering with the immune system's ability to mount general CTL response is to target an activation-specific marker.

Both CD4 positive helper T (T_H) cells and CD8-positive cytotoxic T (T_{CTL}) cells are predominantly produced in the

thymus wherein they undergo both positive and negative selection. Each T cell produced in this organ is unique by virtue of its polymorphic T Cell Antigen Receptor (TCR) that is matched to the resident Major Histocompatibility Complex Class I (MHC I) or Class II (MHC II) proteins for T_{CTL} and T_H cells, respectively. The mature cells that emerge are highly diverse and selected as discriminators of self versus non-self. As these cells migrate to the periphery, they become responsive to peptide antigens presented within the groove of either MHC I or MHC II heterodimers, depending on the T cell type. Under normal circumstances, only the T cell bearing a TCR that appropriately fits to the foreign antigen-bearing cell will become activated. The rest of the T cell population remains quiescent. The activated T cell clonally proliferates, secretes growth factors and cytokines, and aids in the mounting of both humoral as well as cytotoxic immune responses.

The external generation of a "primary" activation signal within a T cell involves a variety of different proteins in addition to the TCR. The CD3 physically associates with the TCR to form an antigen receptor complex. As the T cell activates, the antigen receptor complex physically associates with either CD4 or CD8, depending on the type of T cell, and directly contacts the appropriate MHC molecule. In order to generate a complete proliferative response other secondary signals are required. These "second" signals can be provided by several other pathways such as CD28/B7, CD40/CD40L and/or CD2 (Bierer et al. Ann. Rev. Immunol. 1989 7:579-599; Linsley et al. J. Ex. Med. 1991 173(3):721-730; Grewal, I.S. and Flavell, R.A. Immunol. Rev. 1996 153:85-106). Although the full activation process is not completely understood, it is known that the avidity of the antigen/TCR interaction plays a role in determining the outcome of the final immune response as well as the type of secondary signal sources received by the T cell.

Unlike MHC class II involved in the recognition of antigen helper T cells, MHC I is ubiquitous. It is a transmembrane-spanning heterodimer consisting of a large alpha chain and a shorter protein, known as B-2 microglobulin. The alpha chain is comprised of three immunoglobulin-like subdomains referred to as the α-1, α-2 and α-3 subdomains. The presented antigen is held in a cleft produced by two adjacent helices in the α-2/α-3 subdomains. The T Cell Antigen Receptor directly recognizes the antigen in the context of these helices. CD8 binds to a distal region of the MHC class I. Its binding occurs across the α-1 subdomain of the alpha chain and the β-2 microglobulin.

The CTL response involves an initial clonal expansion process which generates the set of activated CD8-positive T_{CTL} cells. This activated set of cells is responsible for targeted killing of cells bearing the "activating antigen" in the context of MHC I or of detecting and killing cells that do not bear the "self" MHC I. In general, activated/proliferating cells are highly sensitive to the influx of the "complete" set of activating signals. The sudden loss of one or more of the critical signals results in the induction of programmed cell death, known as apoptosis. T cells are particularly sensitive to the regulatory signals that drive the activation forward.

Crystal structure complexes of human CD8 (alpha chain homodimer) bound to MHC I have been described by Gao et al. (Nature 1997 387:630-634). Equivalent murine complexes have also been described by Kern et al. (Immunity 1998 9(4):519-530). From detailed study of these crystal structures, the parameters describing the motion of atoms of the crystal structure [B-factors, which describe anisotropic harmonic motion of individual atoms] have been determined. The B-factors for each of the atoms comprising a single amino acid were averaged to yield an overall value reflecting the motion of that residue. CD8 is a member of the immunoglobulin

superfamily of proteins and, as such, has three looped domains that are analogous to the complimentarity determining regions (CDRs) on an antibody. These are therefore referred to herein as CDR1, CDR2 and CDR3. In the unbound state of an antibody, the CDRs have mobility. When bound by an antigen, their mobility dramatically decreases. Studies in human CD8 demonstrate its CDRs, when bound to MHC I, to also be more stabilized as compared to the unbound protein. In murine CD8 in the bound state, the greatest degree of contact between CD8 and MHC I occurs across the CDR1 and CDR3 domains of the protein. The carboxy terminal half of CDR2 is not in contact with the MHC I.

Based upon this protein surface recognition determination and in conjunction with a visual inspection of the calculated "hard" surface of the protein, five discrete domains on the surface of the CD8 alpha chain have been selected as targets to develop compounds which inhibit detrimental CTL responses. These target sites form a ridge surrounding a channel that abuts the MHC I surface. In fact, it is believed that the binding of MHC I to CD8 creates a unique "binding site" that is not present on either protein alone as a large cavity is created by binding of CD8 to MHC I. Unique surface features on this binding site were used to design templates in the engineering of multiple test compounds or analogs. Topology unique surface patterns were used to design composite peptides as test compounds intended to mimic or interact with these unique surface patterns. By "mimic" it is meant that the compounds present a similar surface and similar pattern of motion to the topology unique surface patterns. By "interact" it is meant that compounds are modeled to fit into spaces or holes in the surface patterns. Test compounds were assayed for their ability to block the generation and killing function of the CD8-dependent CTL response. Test compounds were designed from both the human and murine structures and assayed in species relevant systems.

It was found that synthetic mimicry of and/or interaction with of any of these surface features, whether by means of peptide or organic synthesis, resulted in highly specific antagonists of the activated CD8 complex.

The first site, referred to as the "SHN" ridge is part of the CDR2 of CD8 and is in partial contact with MHC I. This site resides at the top of the CDR2 loop. Ser-58, Ser-59 and His-60 of this site are in direct contact with MHC I. Asn-61 is pointed away from the class I.

A panel of analogs has been designed and synthesized that are intended to mimic features of SHN surface. A list is shown in the following Table 1.

TABLE 1: Analogs to SHN

Code	Analog and SEQ ID NO:
AC8-1	cgSSHNKyc (SEQ ID NO:1)
AC8-3	cSSHNKpc (SEQ ID NO:2)
AC8-5	cYMASSHNKITc (SEQ ID NO:3)
AC8-6	cASSHNKc (SEQ ID NO:4)
SC8-1	SHNKI (SEQ ID NO:5)
SC8-3	SHNK
SC8-2	SHN
SC8-28	^{d}(SHN)KI (SEQ ID NO:5)
SC8-31	^{d}(SHNK)I (SEQ ID NO:5)
SC8-30	^{d}(SHN)K
SC8-29	^{d}(SHN)

Upper case letters in this Table and the following Tables refer to amino acids that are directly taken from the CD8 amino acid sequence, while the lower case letters refer to amino acids that have been artificially introduced to aid in hydrophobicity of the analog or to conformationally restrain the analog. The sequences listed in brackets have had their amino acids rearranged from the original sequence.

This was either done to scramble the analog in some cases or to probe for the importance of the backbone carbonyl and nitrogens in other cases. The amino acids within the parentheses preceded by the superscripted "d" indicate the use of "d" amino acids. The peptides were synthesized as described previously by Jameson et al. (Nature 1994 368:744-746) on an Advanced Chemtech 440 automated organic synthesizer.

All assays used in this study to characterize the analog panels relied on two qualitatively different questions regarding the CTL responses. The first was designed to look at the effects of the analogs on the generation of activated CTLs in the response to an allo-antigen. The second addressed the ability of the test analogs to inhibit the CTL effector functions, i.e. target lysis.

Of the analogs shown in Table 1, only SC8-29 showed reproducible inhibition. SC8-29 is an all "d" amino acid analog consisting of the residues Ser-His-Asn. The all "I" amino acid equivalent (SC8-2) had no activity.

The second surface is referred to as the KIT surface as amino acids lysine, isoleucine, threonine and tryptophan (KITW) were found to be prominently displayed. This portion of the surface is clearly away from the MHC I binding site and flanks a major channel running down the exposed face of the CD8 alpha subunit. The Lys-62 and Thr-64 are used to create the surface of the ridge and Ile-63 and Trp-65 reach down toward the hydrophobic channel. This ridge is directly across the channel from the "RDT" ridge. Several different analogs were designed from this surface regions and are shown in Table 2.

TABLE 2: Analogs to KIT

Code	Analog
SC8-12	KITW
SC8-5	KIT
SC8-37	[ITWK]*
SC8-38	[TIK]
SC8-39	[ITK]

In analyzing this region, it can be seen that the main chain nitrogen arising from Lys-62 and the carbonyl group from Ile-63 have a prominent role in the creation of the surface and serve as potential hydrogen bond donors and acceptors, respectively. Further, the backbone nitrogen and carbonyl of the Trp-65 are also in a position to create a portion of the displayed surface. These hydrogen bond donors and acceptors must be accounted for when designing drugs to target this surface. Synthetic peptide analogs of this ridge, KITW and ITWK, inhibited the CD8 specific response in a dose-dependent manner. Analogs derived from the linear amino acid sequence flanking the KIT ridge including SHN**KI** (SEQ ID NO:5), **W**DEK, and **W**DEK**L** (SEQ ID NO:6), wherein the bolded residues designate amino acids within the KIT ridge, had no effect on CTL response. ITWK was designed as an isoteric analog of KITW. When inverted, the modeled SC8-37 showed remarkable shape similarity to the SC8-12. The tryptophan in ITWK is positioned to mimic the isoleucine following the lysine in the native structure. The critical backbone nitrogens and carbonyls also aligned well to the crystal structure. Modeling studies indicated that the alignment of the ITWK matched the CD8 protein surface better than the KITW peptide. Further, this better alignment corresponded with better inhibitory activity as compared to KITW.

Target sites 3 and 4, referred to herein respectively as DEK and SSK, emanate from a surface region of the CD8 with

the largest associated B-factors. These sites have been consolidated because they represent two halves of the same ridge. Furthermore, an analog spanning both site 3 and 4 (AC8-9) has been reported to inhibit the activation of the CTL response, but not the effector functions (Choksi et al. Nature Medicine 1998 4:309-314). The analog panels synthesized for these sites are shown in Table 3.

TABLE 3: Analogs to DEK and SSK

Site # 3 DEK		Site #4 SSK	
Code	**Analog (SEQ ID NO:)**	**Code**	**Analog (SEQ ID NO:)**
AC8-7	cTWDEKLNc (SEQ ID NO:7)	AC8-8	cDEKLNSSKLFc (SEQ ID NO:10)
AC8-13	cpDEKLNapc (SEQ ID NO:8)	AC8-9	cDEKLNSSKLc (SEQ ID NO:11)
SC8-7	EKL	SC8-17	cSSKc (SEQ ID NO:12)
SC8-13	WDEK	SC8-34	SSK
SC8-14	WDEKL (SEQ ID NO:6)	SC8-25	NSSKL (SEQ ID NO:13)
SC8-15	cDEKc (SEQ ID NO:9)		
SC8-32	DEK		

All of the analogs synthesized from the DEK panel failed to reproducibly inhibit either the generation phase of the CTL response or the cell-mediated killing activities of the CTLs. The SSK panel, on the other hand, exhibited reproducible inhibition of the generation phase of the CTL response, but little, if any, effect on the effector function. The analogs SSK and NSSKL (SEQ ID NO:13) had no effect on the CD8-dependent biological activity, whereas an analog that was conformationally restrained by the artificial introduction of a disulfide bridge to resemble this ridge, cSSKc (SEQ ID NO:12), had full inhibitory activity. Thus, the SC8-17 analog

appears to retain the full biological activity of the larger AC8-9 analog.

The fifth surface or site is referred to as the RDT ridge as amino acids arginine, aspartate and threonine are prominently displayed. This ridge is situated vis-a-vis from the KIT ridge. Parts of this site are close to MHC I, but clearly not in contact with it. An analysis of the B-factors associated with bound MHC I indicate that the α-2 region of MHC that is juxtaposed to the RDT site is one of the most flexible areas of the MHC protein. The region equivalent to the RDT site in unbound CD8 has very little motion associated with it. In the structure bound to MHC I, however, the same ridge shows a significant degree of motion. Thus, the mobility of this ridge is clearly influenced by the binding of MHC. The panel of analogs synthesized to probe this region of the protein is listed in Table 4.

TABLE 4: Analogs to RDT

Code	Analog and SEQ ID NO:
AC8-2	cRDTNNKYc (SEQ ID NO:14)
AC8-10	cpRDTNNgc (SEQ ID NO:15)
SC8-6	NNKYV (SEQ ID NO: 16)
SC8-8	DTN
SC8-9	DTk
SC8-10	TNN
SC8-11	TNNK
SC8-35	T^dNN
SC8-19	cTNNKc (SEQ ID NO:17)
SC8-20	cTNNc (SEQ ID NO:18)
SC8-21	cNNKc (SEQ ID NO:19)
SC8-22	cNNc
SC8-4	RDTN
SC8-33	RDT

SC8-16	cRDTc (SEQ ID NO:20)
AC8-2'	[TDR]
AC8-2"	[cTDRc] (SEQ ID NO:21)
SC8-36	[NTN]

Arg-79 and Asn-83 of this site are both facing in toward the channel and toward the MHC I α-3 domain, whereas Asp-80, Thr-81 and Asn-82 are facing away from the channel and toward the α-2 domain of the MHC. A conformationally restrained peptide encompassing one side of this surface such as cRDTc (SEQ ID NO:20) was demonstrated to have full inhibitory activity, while the unrestrained analog RDTN showed no biological activity. Use of a "*d*" amino acid to conformationally influence the main chain torsions in the analog T(*d*)NN also gave rise to an analog with full inhibitory activity, while the unrestrained TNN peptide had significantly less activity. However, substitutions of the Arg in the cRDTc analog with either Lys, or Gly resulted in compounds with diminished activity. Conservative substitution of the Asp with the same chain length amide moiety (Asn) resulted in only a minor decrease in activity. Similarly, maintaining the acid and lengthening the chain by a methyl group resulted in only a small decrease in activity. Use of a Gln in this position, however, killed all biological activity. Thus, the side chain carboxylic acid clearly contributes significantly to the biological activity of the analog. Finally, with respect to the Thr position, it was found that substitution with either a Ser or a Val resulted in only a marginal loss of activity and substitution with a Tyr at this position improved the overall activity of the analog. Accordingly, a preferred analog targeting the RDT ridge comprises cRDYc (SEQ ID NO:22).

For each surface, it was also found that the backbone nitrogen and carbonyls play an important role in surface presentations. In fact, the analog designed with the

appropriate side chain presentation of the surface, but without the appropriate orientation of the backbone nitrogen and carbonyls (CTDRC; SEQ ID NO:21) retained only a fraction of the biological activity.

Analogs targeted to the RDT ridge were also tested in a murine model designed to determine the ability of these test compounds to induce clonal deletion of only the activated set of CTLs without impacting the ability of the animal to respond to novel antigens. All animals were able to mount strong allo-responses at the end of the study. No animals were immunosuppressed. Further, in the allogeneic response, animals that had been challenged with virus prior to their sacrifice usually showed a slightly stronger alloresponse. In the animals that received the cRDTc (SEQ ID NO:20) analog, which was demonstrated to be a strong inhibitor *in vitro*, the anti-viral response was completely abated. However, even the cys-T-D-R-cys analog, which demonstrated only weak inhibition *in vitro*, showed inhibition of the target-specific response *in vivo*.

A preferred RDT analog, cRDYc (SEQ ID NO:22) was also tested *in vivo* in an accelerated diabetes model in NOD mice. In this model, diabetogenic cells are passively transferred to naive recipients. All control mice developed diabetes before day 19. In contrast, all of the mice treated with cRDYc had a significantly delayed onset of diabetes (< than 2 months).

Accordingly, as demonstrated herein, compounds designed to mimic the topology unique surfaces, SHN, KIT, DEK, SSK and RDT, can be useful in specifically inhibiting detrimental CTL responses in both *in vitro* and *in vivo* assays. Compounds designed to mimic the murine CD8/MHC I surfaces including, but not limited to, those specifically exemplified herein are useful as reagents for enabling vector-driven gene delivery systems wherein detrimental CTL responses present a problem. Such murine derived analogs as described herein or designed

in accordance with methods described herein could be incorporated into kits comprising gene delivery systems for use in research and development of new gene therapies.

Further, these unique surface patterns serve as templates for design of additional compounds which mimic the surface patterns and inhibit detrimental CTL response. Compounds designed from the surface of the human CD8/MHC I complex to mimic unique surface feature specifically inhibit detrimental CTL responses and are thus useful in treating autoimmune diseases including, but not limited to IDDM, rejection from alloengraftments, rejection occurring in Graft versus Host disease and solid organ transplantation. Further such compounds are useful in inhibiting the detrimental CTL response which currently poses a potential limitation to the development of gene therapies in humans.

In the human CD8-MHC complex, the focus has been on the β-2 microglobulin domain of the MHC. The β-2 microglobulin loop corresponds to MHC residues Asn-83, His-84, Val-85, Thr-86, Leu-87, Ser-88 and Gln-89. The LSQ portion of this loop provides part of the edge of the cavity created when CD8 binds to MHC. The NHVT portion of the loop faces away from the CD8 surface. Several small peptides from this loop were synthesized and tested in a human CTL effector assay. The analogs LDT (a peptide similar in sequence to the active analog) NHVT, HVT and LSQ were assayed for their ability to inhibit human CTL target lysis responses. The analogs were assayed at 200, 100 and 20 μg/ml. The LSQ analog inhibited about 25 to 30% of the response. Peptides derived from the side of the MHC loop facing away from the CD8 surface had no activity. These data are consistent with the belief that the larger cavity created by the binding of CD8 to MHC serves as a protein recognition site for the physical association of other proteins.

Similar approaches have been used to map the exposed surface of the MHC-beta subunit in this bound protein complex.

It is believed that the T cell antigen receptor complex physically associates with the β-monomer of the αβ heterodimer. The beta chain was modeled using the murine homodimer bound to MHC as a template. The selection of which subunit to modify with the side chains of the β-subunit was determined via the contact of Arg-8 with MHC. Only one of the α-subunits contacts MHC via Arg-8. This subunit also had the greatest surface contact area with the MHC. Consequently, the other subunit was used as the β-chain template. The β-chain was modeled using coordinates of the murine CD8-MHC complex and sequence alignment was performed. The β-specific side chain replacements on the CD8 template were then used to create the new model of the αβ heterodimer as described by Jameson (Nature 1989 341:465-467) and Jameson et al. (Nature 1994 368:744-746). After replacing the side chains, the model was subjected to alternating rounds of molecule motions. The modeling was performed using the Biopolymer module from the Sybyl computational chemistry suite of programs on a Silicon Graphics OCTANE computer. A Connolly Surface was calculated from the NMR-based structure using a hypothetical sphere with a radius of 2.8 Å (twice the radius of a water molecule). An electropotential gradient was superimposed on the surface of the protein to highlight the highest and lowest electropotentials.

Five ridges have been identified on the surface of the CD8 β-chain. The first, NHT, is centered around residues 12-17, TNHTAK (SEQ ID NO:23), and is partially comprised of the adjacent residues 83-87, IMNVK (SEQ ID NO:24). The second ridge is a segment of the CDR1, comprised of EVKSISK (21-27l SEQ ID NO:25). The third ridge is the most prominent (and the most highly charged) consisting of the amino acids SVDKKRN (62-68; SEQ ID NO:26). The fourth ridge (LES) displays residues IILES (69-73; SEQ ID NO:27). The fifth ridge (SDS) is in a position equivalent to the RDT ridge on the α-chain. This prominently displayed surface is comprised of residues

SSDSRRPFL (73-81; SEQ ID NO:28). The design of analogs to these ridges was performed in analogous fashion to analogs designed and described herein.

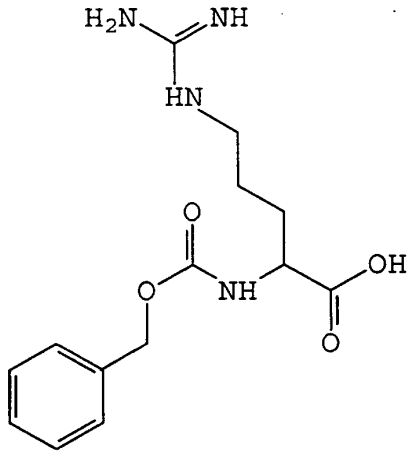
Several hybrid CD8 β-chain mimetics which constitute an adjacent surface derived from a discontinuous sequence were also designed and synthesized. Arg-78, Glu-21 and Val-22 are spatially very close to one another in the CD8 β-subunit. It has been found that a proline inserted between the Arg and Glu-Val provides the appropriate geometry of presentation. The activity of analogs of the CD8 β-subunit in cytotoxicity assays is shown in Figure 1.

In addition to designing analogs to mimic the surface of the CD8 β-chain, several analogs intended to interact with the β-chain surface were also designed. Solid surface representations of CD8 showed a small hole in the surface domain with a depth and width suitable for insertion of compound comprising an aromatic ring. This hole is in the surface adjacent to the "SDS" region. Accordingly, various analogs were modeled to the fit the hole. In each case, the aromatic ring was fit in the hole and the surrounding surface was analyzed for potential hydrogen bonds. It was found that semi-organic compounds, such as carbobenzoxy (cbz) arginine, gave a relatively good fit as did the dipeptide Arg-Phe. The inverse sequence Phe-Arg was predicted to lack several of the hydrogen bonds observed with the Arg-Phe peptide. In assays depicted in Figure 1, a dose-dependent inhibitory response was observed for both the Arg-Phe peptide and the semi-organic compound cbz-arginine. In contrast, as predicted by its fit in modeling analysis, the Phe-Arg analog has no activity. See Figure 1. Based upon these experiments, it is believed that other semi-organic compounds comprising a simple aromatic ring can be designed to interact with a surface feature of human CD8 specific to the interaction of human CD8 with MHC I thereby inhibiting a detrimental T lymphocyte response. Pharmaceutical compositions comprising semi-organic compounds

such as cbz-arginine or peptide mimetics such as described herein can then be prepared and administered to humans in accordance with well known techniques to inhibit detrimental T lymphocyte responses including, but not limited to, those responses which limit the effectiveness of gene therapies and tissue transplantation and which occur in patients with insulin-dependent diabetes mellitus (IDDM).

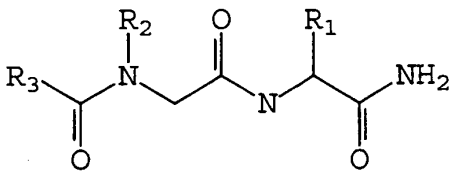
The structure of carbobenzoxy (cbz) arginine is depicted below as Formula I:

(I)



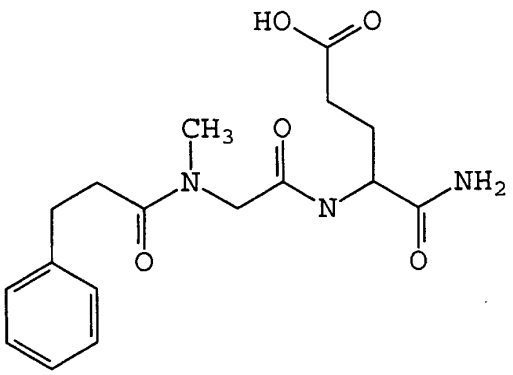
This compound contains an ester linkage predicted by the computer model to have no biological purpose. However, this linkage is quite likely to limit the utility of cbz-arg *in vivo*. Accordingly, a series of semi-organic analogs without the ester linkage, but with a similar basic structure as depicted in Formula II (as shown below), have been synthesized and tested for inhibitory activity.

(II)

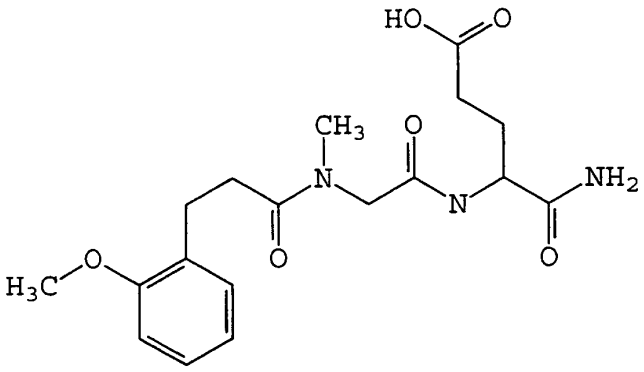


Specific examples of analogs with this basic structure tested for inhibitory activity are depicted in Formula III-VII.

(III)



(IV)



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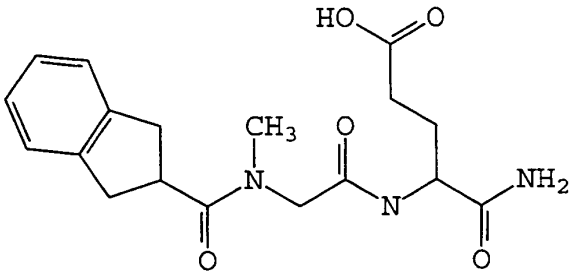

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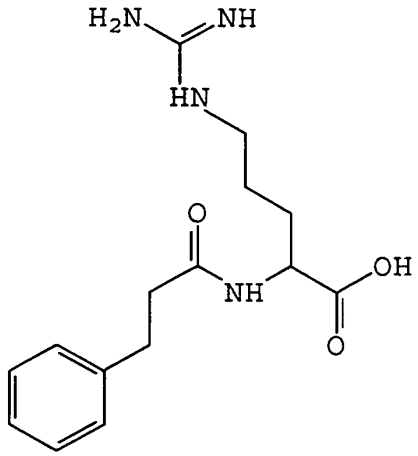

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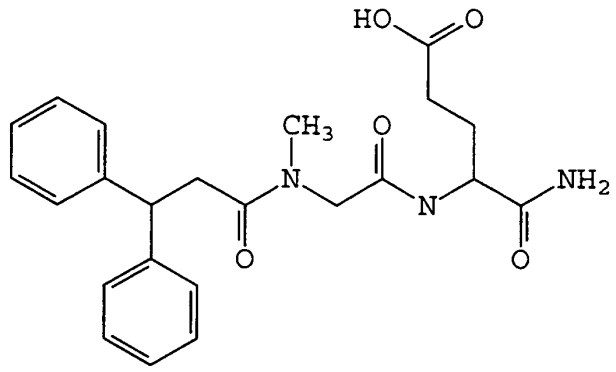

Of these, the analog of Formula VII is preferred. As will be understood by those of skill in the art upon reading this disclosure, however, additional analogs which interact with unique surface features of the CD8/MHC I complex wherein R_3 comprises one or more ring structures comprising 4 to 8 carbons and R_1 and R_2 have been modified to further enhance activity can also be produced combinatorially and used to inhibit detrimental Cytotoxic T lymphocyte responses.

The following nonlimiting examples are provided to further illustrate the present invention.

EXAMPLES

Example 1: Murine CTL Response Assay

CTL response assays were performed to examine the effects of analogs on the generation of activated CTLs in response to an alloantigen and the ability of the analogs to inhibit CTL effector functions such as target lysis.

For the general CTL assay, the primary spleen cells from C57BL/J6 mice were stimulated with mitomycin-treated irradiated (10,000 RADs) P815 cells at a 6:1 effector to stimulator ratio. Stimulated spleen cells were then cultured in RPMI media supplemented with human recombinant IL-2 (1 U/ml), 10% FCS, glutamic acid, penicillin and streptomycin. After 6 days in culture, the allogeneic CTL response was assayed in accordance with the protocol described by Matzinger (J. Immunol. Meth. 1991 145:185-192). The effector cells were incubated with the [^{3}H]labeled targets (P815 cells) at a 50:1 ratio for 3 hours at 37°C and harvested using a PHD harvester. The percent of specific killing was determined using the formula:

% killing = (S-E)/S,

where S is the amount of the DNA retained by the target cells in the absence of the effector cells and E is the amount of retained DNA in the presence of the effector cells (in cpms).

To assay for the effect of the peptides on the generation and effector phases of the CTL response, spleen cells were treated with the peptides either at the time of the stimulation or at the time of the killing assay. To rule out the possibility of the toxic effects of the peptides, [^{3}H] incorporation by P815 cells that were cultured in the presence of the peptides for 3 days was determined. None of the peptides showed any signs of toxicity.

Example 2: *In vivo* CTL response in Mice

Murine studies were performed to determine whether the engineered inhibitory analogs could induce clonal deletion of

only the activated set of CTLs without impacting the ability of the animal to respond to novel antigens. In order to induce a well characterized CTL response, the murine retrovirus MuLV was inoculated (1 x 10^5 FFU) into C57BL/6 mice on day 0 of the study. Under these conditions, it is known that C57BL mice create a strong CTL response directed solely at the E55 envelope protein of the virus. Following the initial viral inoculation, the mice were allowed to develop a CTL response. This response typically takes 5 to 7 days. One day 9 of the study, a single bolus (i.v.) injection of the test analog was administered. Small hydrophilic analogs such as peptides used in this study are generally very rapidly removed from the animal via renal clearance mechanisms. With a short serum half-life and an observed IC_{50} of the mid-lower micromolar range, it is reasonable to assume that an inhibitory effect would only be seen at the end of the study if the analog can induce severe allergy or, more likely, clonal deletion of the activated CTLs. The anti-viral CTL response was boosted by a re-challenge of MuLV on day 11. Because the CTL response to re-challenge is very rapid, a second bolus injection of the analog was administered on day 13. The mice were sacrificed on day 21 of the study. The CD8 positive cells of each animal are split into aliquots. The first was used to assay for the virus-specific response to E55-positive target cells. The second was used to show that the resting repertoire is fully functional by stimulating an allogeneic CTL response. For this study, 5 groups of three C57BL/6 mice each were used as described in the following table.

Group (N=3)	Virus Inoculation	Analog Used	Analog Sequence	Analog Activity (*in vitro*)
C-	none	none		
C+	+	none		

P-	+	SC8-29	S-H-N	Control
P+/-	+	SC8-2	cys-T-D-R-cys	Weak inhibition
P+	+	SC8-16	cys-R-D-T-cys	Strong inhibition

Example 3: Accelerated Diabetes Murine Model

Lethally irradiated, two month old, female NOD mice received 10^7 spleen cells from donor diabetic mice. Two days prior to passive transfer of t he spleen cells, the test analog cRDYc (SEQ ID NO:22) was inoculated into the donor diabetic female NOD mice. The spleen cells from the donors were split into 2 groups. One group was left untreated and used in control animals. The second group was incubated for 20 minutes with 200 μg of the cRDYc analog prior to transfer into the animals. Treated animals also received 400 μg of the analog intravenously at approximately days 3 and 7 post transfer of the spleen cells.

Example 4: Human CTL assay

Human blood PBL cells from the first donor (effector) were stimulated with irradiated (3,500 RADs) PBL cells from a second donor (stimulators) or with irradiated Sup-T1 cells (10,000 RADs) at 6:1 effector to stimulator ratio. Stimulated blood cells were cultured in RPMI media supplemented with human recombinant IL-2 (1 u/ml), 10% FCS, glutamine, penicillin and streptomycin. After 7 days in culture the CTL response was assayed using a protocol developed by Matzinger (Immunological Methods 1991 145:185-192). These effector cells were incubated with the [^{3}H] labeled targets (Sup-T1 cells or PBL cells from the second donor grown in the presence of 1 μg/ml conA) at 50:1 ratio for 3 hours at 37°C and harvested using a PHD harvester. Percent specific killing was determined using the formula

% killing = (S-E)/S,

where S is the amount of DNA retained by the target cells in the absence of the effector cells and E is the amount of DNA retained in the presence of the effector cells (in cpms).

To assay for the effect of the peptides on the generation and effector phases of the CTL response, human peripheral blood cells were treated with the peptides at the time of the killing assays.